

Function of the Dufour's gland in solitary and social Hymenoptera

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Abstract

The poison gland and Dufour's gland are the two glands associated with the sting apparatus in female Apocrita (Hymenoptera). While the poison gland usually functions as an integral part of the venom delivery system, the Dufour's gland has been found to differ in its function in various hymenopteran groups. Like all exocrine glands, the function of the Dufour's gland is to secrete chemicals, but the nature and function of the secretions varies in different taxa. Functions of the Dufour's gland secretions range from serving as a component of material used in nest building, larval food, and pheromones involved in communicative functions that are important for both solitary and social species. This review summarizes the different functions reported for the Dufour's gland in hymenopterans, illustrating how the Dufour's gland secretions can be adapted to give rise to various functions in response to different challenges posed by the ways of life followed by different taxa. Aspects of development, structure, chemistry and the evolution of different functions are also touched upon briefly.

Keywords

Dufour's gland, function, structure, chemistry, evolution, Hymenoptera

Introduction

The poison gland and Dufour's gland are the two glands associated with the sting apparatus in female Apocrita (Hymenoptera) (Fig 1). The Dufour's gland is generally found in most Apocrita, and in Symphyta a homologous gland may exist (Robertson 1968, Van Marle and Piek 1986).

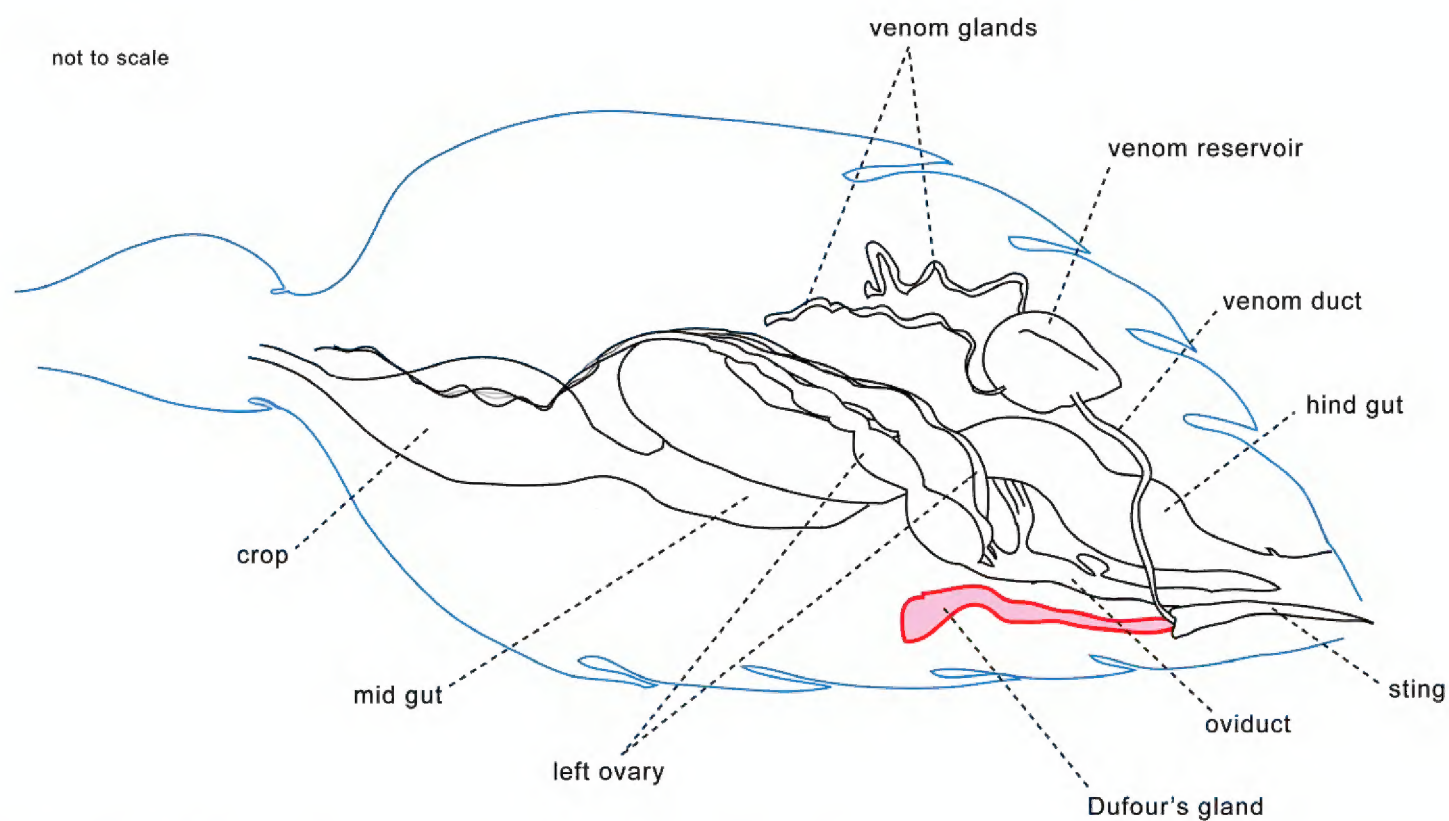


Figure 1. Diagram showing position of the Dufour's gland among other abdominal organs in the polistine wasp *Ropalidia marginata*. (Drawing: author, not to scale)

Hymenopterans probably originated during the Triassic period (about 250 to 200 million years ago), and the oldest fossils found are similar to sawflies of the family Xyelidae (Symphyta) (Rasnitsyn 2002). The Hymenoptera started diversifying during the Cretaceous (about 150 to 70 million years ago), and this diversification took place simultaneously with other major insect groups like the Lepidoptera, Coleoptera and Diptera, in conjunction with the rise of the flowering plants, signifying plant-insect coevolution (Hoell et al. 1998, Grimaldi and Engel 2005). The first social Hymenoptera are also believed to have evolved in the Cretaceous. Although the establishment of the major hymenopteran categories like Apocrita, Aculeata and non-aculeate Apocrita, and the subsequent split between ancestors of present day aculeates (Apoidea, Vespoidea and Chrysidoidea) appears to have taken place earlier, in the Jurassic (about 200 to 150 million years ago) (Rasnitsyn and Zhang 2010, Heraty et al. 2011), much of the diversification appears to have taken place in the Cretaceous, when the major Aculeate clades became established (Brady et al. 2009). Thus the Dufour's gland may have been evolving for over more than 200 million years to give rise to the variety of functions it exhibits in modern hymenopterans.

While the poison gland usually functions as an integral part of the venom delivery system, used for paralyzing prey to be used as food as well as in defense, the Dufour's gland has been found to differ in its function in various hymenopteran groups. Like all exocrine glands, the function of the Dufour's gland is to secrete chemicals, but the nature and function of the secretions varies in different taxa. Functions of the Dufour's gland secretions range from serving as a component of material used in nest building, larval food, and various communicative functions like host marking, nest recognition, nestmate recognition, kin recognition, fertility signaling, as a trail pheromone and as a

sex pheromone. These are important for both solitary and social species. The purpose of this review is to summarize the different functions reported for the Dufour's gland secretions in hymenopterans. This will further the understanding and appreciation of how an organ can be adapted for different functions in different lineages, and how differences in the mode of living has led to an organ being adapted for diverse functions. Aspects of development, structure and chemistry are also touched upon briefly.

Development and structure

The Dufour's gland (often referred to as "alkaline gland" in the older literature) was first described by Dufour (1841). It is an ectodermal abdominal gland located near the proximal end of the abdomen (Fig. 1), and is believed to be a homologue of the colleterial gland of other insects, i.e. an accessory reproductive gland (Carlet 1884, Maschwitz and Kloft 1971, Hermann 1969, Hermann and Blum 1967a, 1967b, 1981, Abdalla and Cruz-Landim 2001). The sting apparatus in Hymenoptera is ectodermal in origin and arises as a modification of the female reproductive system, as evidenced from developmental ontogeny. Buds of genitalia develop on the 8th and 9th abdominal sterna of hymenopteran larvae, and these develop into the ovipositor (sting). The spermatheca, poison gland and Dufour's glands develop as invaginations of valves of the sternum (D'Rozario 1942, Robertson 1968). The duct of the Dufour's gland opens into the sting in ants, while in wasps and bees it opens into the dorsal vaginal wall. The duct opening into the vaginal wall has been proposed as the ancestral state, while opening into the sting has been proposed as a derived state (Billen 1987). However, in a digger wasp, it has been reported that the Dufour's gland opens into the sting, thereby raising doubts about the earlier hypothesis (Gnatzy et al. 2004).

The morphology of the Dufour's gland can vary between groups, but the structure is essentially a single layered epithelial tube. It is partially covered by a muscular layer and is associated with tracheoles and nerves (Hermann and Blum 1967a, 1967b, Landolt and Akre 1979, Downing and Jeanne 1983, Billen 1986a, 1986b, Abdalla and Cruz-Landim 2004, Abdalla et al. 1999). Since there is a single layer of glandular epithelium, the Dufour's gland is a "class I" epidermal gland, as described by Noirot and Quennedy (1974). The gland cells are abundant in smooth endoplasmic reticulum, secretory vesicles, mitochondria, and electron dense granules, consistent with their role in secretion (Billen 1982, 1986a, 1986b). The cells secrete material into the lumen of the gland, which is then carried outwards. Muscles located around the opening of the gland's duct may help in controlling the flow of secretion.

Chemistry

The Dufour's gland is believed to have evolved from the colleterial gland. Therefore in the ancestral state, the Dufour's gland secretions might be similar to colleterial gland

secretions, found to be glucosides, proteins and enzymes in Lepidoptera and Blattodea, consistent with its role in reproduction (Pau et al. 1971, Sugumaran and Nellaiappan 1990, Jin et al. 2006). Unfortunately the chemistry of the colleterial gland/Dufour's gland in basal hymenopterans (Symphyta) and in many other non-aculeate hymenopterans remains unexplored. The Dufour's gland secretion has generally been found to contain long chain saturated and unsaturated hydrocarbons in most taxa that have been examined. In addition to hydrocarbons, various other volatile, semi-volatile and non-volatile compounds like terpenoids, alcohols, esters, long chain fatty acids, sugars, quinones, various aromatic compounds, traces of cholesterol and aldehydes have been reported. Fig. 2 lists the types of compounds that have been reported from different groups of hymenopterans.

It can be seen that secretion of long chain hydrocarbons is the most ubiquitous trait amongst all hymenopterans whose Dufour's gland chemistry has been analyzed (Fig. 2). Thus secretion of hydrocarbons is likely to be an ancestral trait common to all hymenopterans, while other categories of compounds that have been reported are likely to be derived traits. In Apoidea, Andrenidae, Colletidae and Halictidae are closely related clades that share esters with terpene derivatives (Hefetz et al. 1979, Duffield et al. 1981, Hefetz 1987). The Colletidae and Halictidae are sister clades that share macrocyclic lactones. The Megachilidae is sister to the Apidae and has triglycerides, a trait shared with Anthophorini, which is basal to other apids (Cane 1981, Cane and Carlson 1984, Kronenberg and Hefetz 1984, Cardinal et al. 2010). This trait is absent in other apids, who share esters as a common trait, suggesting that ester production may be a trait that evolved early during the diversification of the Apidae (Vinson et al. 1978, Duffield et al. 1984, Kronenberg and Hefetz 1984, Katzav-Gozansky et al. 1997, Abdalla et al. 2004, Amsalem et al. 2009). In the Vespoidea, again hydrocarbons are the trait common to most taxa, while the Formicidae show the presence of categories of compounds not found in other vespoids (see Fig. 2). Since the Formicidae show considerable adaptive radiation and occupy diverse ecological microhabitats, it is not surprising to see several derived traits with respect to Dufour's gland chemistry in the Formicidae. Hydrocarbons are likely to be the conserved trait in Dufour's gland chemicals of Hymenoptera, and different clades show appreciable differences in the composition of their Dufour's gland compounds, with the majority of clades presenting an admixture of ancestral and derived traits.

Function

The Hymenoptera form a basal sister clade with respect other insect orders like the Lepidoptera, Trichoptera, Siphonaptera and Diptera (Kristensen 1981, Whiting 2002, Kjer 2004). Since the Dufour's gland is believed to have evolved from the colleterial gland (an accessory reproductive gland) that is present in other insects (Carlet 1884, Maschwitz and Kloft 1971, Hermann 1969, Hermann and Blum

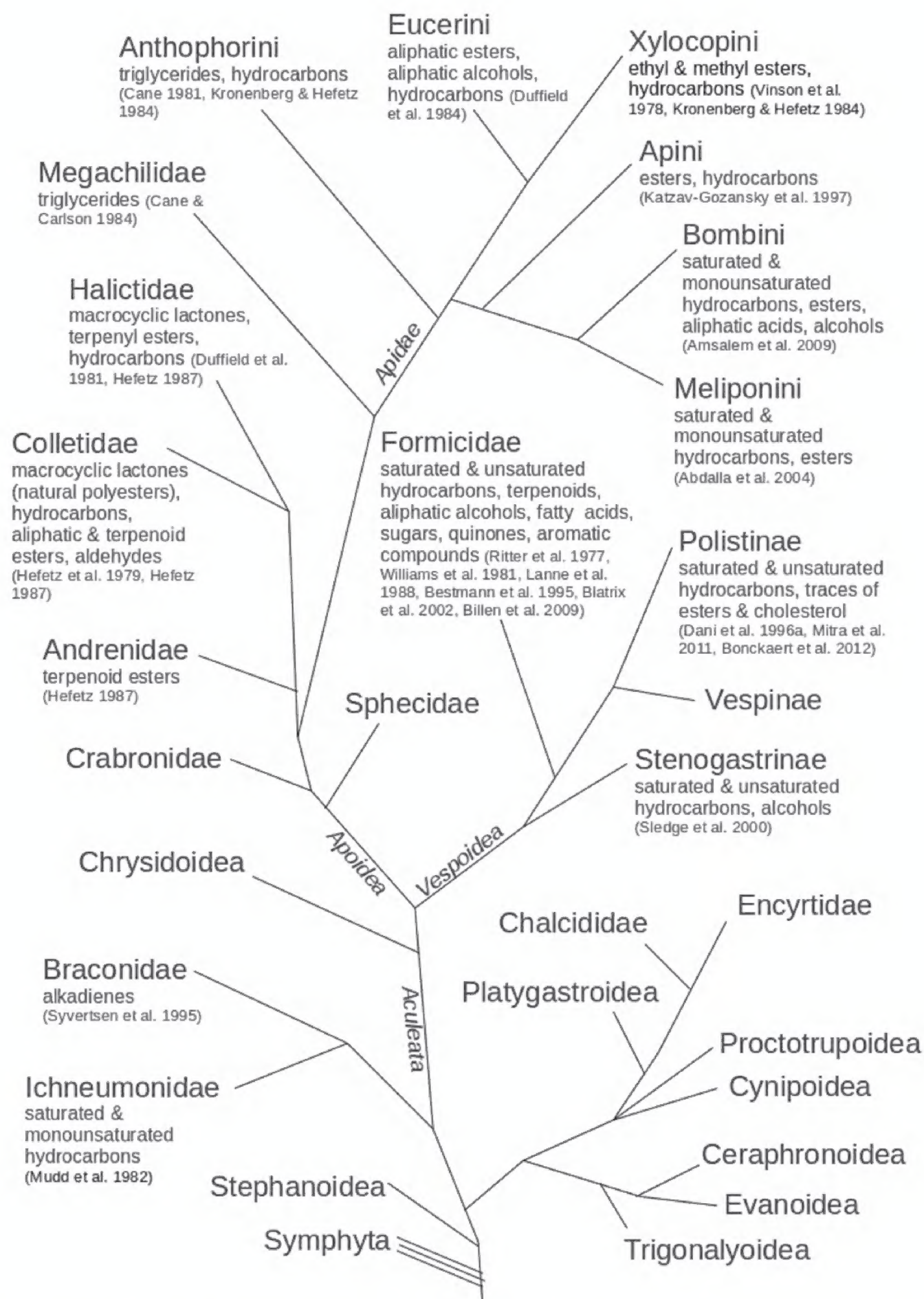
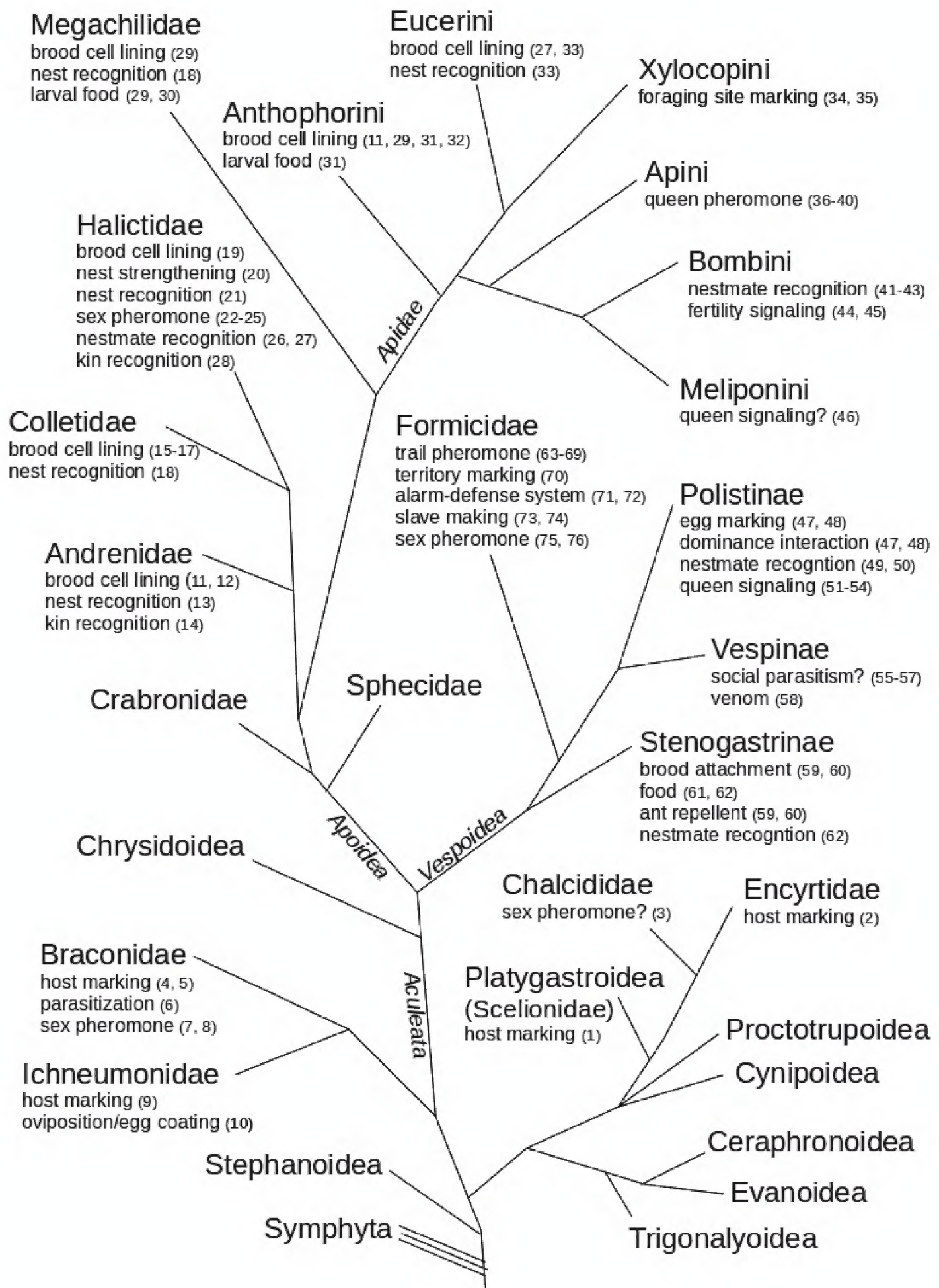


Figure 2. Diagram listing the categories of compounds reported from the Dufour's gland in various hymenopteran taxa, also showing the inter-relationships of the taxa. Dendrogram made following Brothers (1998), Schmitz and Moritz (1998), Whitfield and Cameron (1998), Grimaldi and Engel (2005), Brady et al. (2009), Vincent et al. (2010), and Cardinal et al. (2010). References cited in parenthesis below the names of compounds. Branch lengths do not have any significance.

1967a, 1967b, 1981, Abdalla and Cruz-Landim 2001), the ancestral function of the gland could be similar to roles performed by the colleterial gland in other insects, like forming a protective casing (ootheca) over the eggs, or producing cementing material for attaching eggs to the substrate (Brunet et al. 1952). Colleterial glands in a moth (Lepidoptera, a sister clade of Hymenoptera) have been found to function in attaching eggs to leaves of plants (Chaudhuri and Sinha 1994). The colleterial gland in Symphyta has been reported as the homologue of the Dufour's gland (Robertson 1968, Van Marle and Piek 1986) but unfortunately, the function of this gland in symphytans (basal Hymenoptera) remains unexplored (Fig 3). With the exception of Orussidae, which is parasitic and basal to the apocritans, other symphytans are phytophagous, with Xyelidae being the clade basal to all other Hymenoptera (Davis et al. 2010). Perhaps the colleterial gland in Symphyta may be involved primarily in cementing eggs to plants, as in other phytophagous insects. In the older literature the Dufour's gland was often referred to as the "alkaline gland" and its secretions were assumed to be alkaline in nature in contrast to the poison gland, whose secretions were deemed acidic. It was often assumed that the general function of the Dufour's gland secretion is to lubricate the sting or ovipositor, lubricate passage of eggs during oviposition, glue eggs to the substratum, or to provide a protective coating to eggs, but experimental evidence for these postulated functions is absent (Dufour 1841, Billen 1987, Howard and Baker 2003, Gnatzy et al. 2004). The Dufour's gland is reported to serve various communicative purposes in social apocritans (Fig. 3).

Figure 3. Diagrammatic summary of the known functions of the Dufour's gland in various hymenopter-an taxa, also showing the inter-relationships of the taxa. Dendrogram made following Brothers (1998), Schmitz and Moritz (1998), Whitfield and Cameron (1998), Grimaldi and Engel (2005), Brady et al. (2009), Vincent et al. (2010), and Cardinal et al. (2010). Figures in parenthesis beside each function denote references cited. Branch lengths do not have any significance.

References: **1** Rosi et al. (2001), **2** Mehrnejad and Copland (2007), **3** Vardal (2006), **4** Guillot et al. (1974), **5** Vinson and Guillot (1972), **6** Lawrence and Akin (1990), **7** Vinson (1978), **8** de Freitas et al. (2004), **9** Marris et al. (1996), **10** Huang et al. (2008), **11** Cane (1981), **12** Duffield et al. (1983), **13** Hefetz (1987), **14** Ayasse et al. (1990b), **15** Hefetz et al. (1979), **16** Albans et al. (1980), **17** Cane (1981), **18** Guedot et al. (2006), **19** Cane (1981), **20** Brooks and Cane (1984), **21** Ayasse et al. (1990a), **22** Smith et al. (1985), **23** Barrows (1975a), **24** Barrows et al. (1975b), **25** Ayasse et al. (1993), **26** Hefetz et al. (1986), **27** Hefetz (1987), **28** Smith and Wenzel (1988), **29** Cane and Carlson (1984), **30** Duffield et al. (1984), **31** Norden et al. (1980), **32** Hefetz et al. (1982), **33** Shimron et al. (1985), **34** Frankie and Vinson (1977), **35** Vinson et al. (1978), **36** Katzav-Gozansky et al. (2002), **37** Sole et al. (2002), **38** Oldroyd et al. (2002), **39** Katzav-Gozansky et al. (2007), **40** Malka et al. (2008), **41** Tengö et al. (1991), **42** Oldham et al. (1994), **43** Ayasse et al. (1999), **44** Amsalem et al. (2009), **45** Amsalem and Hefetz (2010), **46** Abdalla and Cruz-Landim (2004), **47** Downing and Jeanne (1983), **48** Downing (1991), **49** Dani et al. (1996a), **50** Dani et al. (1996b), **51** Mitra et al. (2011), **52** Mitra and Gadagkar (2011), **53** Mitra and Gadagkar (2012a), **54** Mitra and Gadagkar (2012b), **55** Jeanne (1977), **56** Reed (1982), **57** Reed and Akre (1982), **58** Barr-Nea et al. (1976), **59** Sledge et al. (2000), **60** Fortunato and Turillazzi (2012), **61** Turillazzi (1989), **62** Cervo et al. (2002), **63** Law et al. (1965), **64** Hölldobler and Wilson (1970), **65** Williams et



al. (1981), **66** Blatrix et al. (2002), **67** Jeanson et al. (2003), **68** Greene and Gordon (2007), **69** Morgan (2009), **70** Cammaerts et al. (1977), **71** Wilson and Regnier (1971), **72** Whitehouse and Jaffe (1996), **73** Topoff et al. (1988), **74** Mori et al. (2000), **75** Hölldobler and Wilson (1990), **76** Ayasse et al. (2001).

a) Parasitic wasps

The evolution of the parasitic life history has been considered to be an important factor that led to the massive divergence and adaptive radiation of the Hymenoptera (Heraty et al. 2011). Unfortunately, the function of the Dufour's gland remains poorly explored in parasitic Hymenoptera that are basal to the more advanced groups of aculeates. Among various non-aculeate parasitic apocritans the function of the Dufour's gland may range from host marking (oviposition site marker), sex attractant, coating the egg surface or facilitating oviposition. In Chalcidoidea, the Dufour's gland region has been found to elicit a male courtship response in the chalcid wasps *Brachymeria lasus* and *B. intermedia* (Chalcididae) and thereby it has been proposed that Dufour's gland secretions may act as a sex pheromone (Simser and Coppel 1980). There is some evidence of Dufour's gland secretion acting as host marking chemicals in *Psyllaephagus pistaciae* (Encyrtidae) that can be used to avoid ovipositing in a parasitized host (Mehrnejad and Copland 2007). The function of Dufour's gland remains unexplored in fig wasps (Agaonidae). In Ichneumonoidea, Dufour's gland hydrocarbons have been implicated in host marking in *Cardiochiles nigriceps* and *Micropletis croceipes* (Braconidae) (Guillot et al. 1974, Vinson and Guillot 1972), and also in *Nemeritis canescens* and *Itoplectis naranyae* (Ichneumonidae) (Mudd et al. 1982, Hubbard et al. 1987, Ueno 1994). It has been found that not only may the Dufour's gland function in host marking in *Venturia canescens* (Ichneumonidae), but females may also discriminate between hosts parasitized by other closely related females versus those parasitized by unrelated females, thereby ensuring that their offspring do not have to compete with other genetically similar larvae in the same host (Marris et al. 1996). In *Diadegma semiclausum* (Ichneumonidae), Dufour's gland secretions have been reported to be added along with venom gland secretions to mature eggs that are ready to be laid (Huang et al. 2008). The Dufour's gland has been reported to act as a female sex pheromone in *Cardiochiles nigriceps* and *Cotesia flavipes* (Braconidae) (Vinson 1978, Syvertsen et al. 1995, de Freitas et al. 2004). In *Biosteres longicaudatus* (Braconidae), virus-like particles have been reported to accumulate in the Dufour's gland, which are injected into the host during parasitization, and may serve in suppressing the immune system of the host during development of parasitic larvae in the host's body (Lawrence and Akin 1990). In Platygastroidea, evidence for host marking has been found in *Trissolcus basalis* (Scelionidae) (Rosi et al. 2001).

The function of the Dufour's gland remains to be studied in Cephrenoidea, Evanioidea, Megalyroidea, Mymarommatoidea, Proctotrupoidea, Stephanoidea, and Trigonalioidea, while recent evidence suggests that the Dufour's gland may be absent in Cynipoidea (Vardal 2006).

b) Aculeata

All social hymenopterans belong to the Aculeata, a clade which has received much attention over the years. Consequently, most of the information on the function of the

Dufour's gland comes from aculeates. Aculeates are characterized by the development of the stinging apparatus, which leads to a separation of the sting from any oviposition related functions (Hunt 2007). In aculeates, the speculations regarding ancestral functions of the Dufour's gland include sting lubrication, venom constituent or role in oviposition (Dufour 1841, Billen 1987, Howard and Baker 2003, Gnatzy et al. 2004), but definitive demonstrations are lacking. Many studies have looked at the function of the Dufour's gland in the two well studied groups: the Apoidea and Vespoidea. Among solitary aculeates, the function of Dufour's gland remains unknown in the Chrysidoidea (cuckoo wasps). Since this group is basal to other aculeates, it would be of interest to investigate the role of the Dufour's gland in this group. Since the Chrysidoidea is comprised mostly of parasitoids, perhaps the role of the Dufour's gland here is similar to that in the parasitoid non-aculeates. The Dufour's gland probably underwent further modifications that were possible with the development of the stinging apparatus of the Aculeata, which removes any constraints related to oviposition from the stinging apparatus (Whitfield 1992), and thereby with the evolution of the sting, the Dufour's gland can be freed from prior evolutionary constraints. Indeed, structural modifications whereby the Dufour's gland no longer remains connected to the reproductive tract but opens instead into the sting has been documented (Billen 1987, Gnatzy et al. 2004). The function of the Dufour's gland in the Aculeata varies widely with variation in mode of living.

In Apoidea, the primary role of the Dufour's gland in various ground nesting bees appears to be producing the brood cell lining. Dufour's gland secretions have been found to be involved in forming a protective lining of the brood cells in various ground dwelling bees in Colletidae, Andrenidae, Halictidae, and some Apidae (Anthophorini and Eucerini), and sometimes they also form a hydrophobic lining for their pollen balls (Hefetz et al. 1979, Albans et al. 1980, Cane 1981, Duffield et al. 1981, 1983, Cane and Carlson 1984, Hefetz et al. 1982, 1986, Hefetz 1987). Ground dwelling bees face the challenge of keeping their larval food free from microbial growth, desiccation, and soaking by water. They have evolved to respond to this challenge by coating their brood cell with a hydrophobic lining that forms a sheath around the larval food. Secretions of the Dufour's gland have been found to be involved in forming this cell lining. Consequently the Dufour's gland has been found to become hypertrophied with the increase in activity during the nest construction phase (Lello 1971a, 1971b, Kronenberg and Hefetz 1984). Production of brood cell lining is an important adaptation for mass provisioners, ensuring that the food remains fresh and suitable for consumption by the larvae. Unfortunately, it is not known whether a similar function is served by the Dufour's gland in ground dwelling digger wasps, sand wasps and bee wolves (Sphecidae and Crabronidae), which utilize similar nest substrates. In the Crabronidae there is evidence for the post pharyngeal gland being involved in prey embalming, which may serve the same function as served by the protective hydrophobic cell lining of ground dwelling bees (Strohm et al. 2001). In the melittid bee *Hesperapis* the Dufour's glands are tiny and appear to be secondarily derived, as they are correlated with the absence of cell lining or with producing cell lining from

exogenously derived substances (Cane 1983). In communally nesting mining bees of genus *Andrena* (Andrenidae), Dufour's gland compounds have been found to vary with genetic relatedness, thereby implying a possible role in kin recognition (Ayasse et al. 1990b). Such compounds have also been implicated in marking the nest entrance, helping in locating the nest within the nesting site (Hefetz 1987). In the sweat bee *Lasioglossum malachurum* (Halictidae), the pattern of Dufour's gland compounds was found to differ between virgin and mated queens, suggesting a possible involvement in mating and courtship (Ayasse et al. 1993). Mated queens had higher proportion of macrocyclic lactones (used in nest construction), and lower proportion of isopentyl esters (volatiles), as compared to virgin queens, and the composition of Dufour's gland compounds was found to change within one day of mating (Ayasse et al. 1993). In the same species, *L. malachurum* (Halictidae), the pattern of ester composition has also been found to differ between young virgin queens and old nesting queens, and the pattern has also been correlated with volume of fat body and status of ovarian development, implying possible involvement in sex pheromonal communication and in nest recognition (Ayasse et al. 1990a). Dufour's gland lactones have been found to act as a sex pheromone in *L. zephyrum* (Smith et al. 1985, Barrows 1975a, Barrows 1975b). Dufour's gland compounds have been implied in nestmate recognition and kin recognition as well in halictine bees (Hefetz et al. 1986, Hefetz 1987, Smith and Wenzel 1988, Soro et al. 2011). In *Halictus hesperus* (Halictidae) Dufour's gland secretion has been found to impart structural strength to the opening of the nest (Brooks and Cane 1984). In *Eucera palestinae* (Apidae: Eucerini), in addition to serving as a cell lining, Dufour's gland compounds also serve to mark the nest entrance and are used by individuals to locate their nest within dense nest aggregations (Shimron et al. 1985). A similar function has been proposed for *Colletes cunicularius* (Colletidae) and *Osmia lignaria* (Megachilidae) as well (Guédot et al. 2006). To serve as a semiochemical used in nest recognition, the Dufour's gland chemicals must show considerable inter-individual variability. Such variability has indeed been reported in some species (Kronenberg and Hefetz 1984, Hefetz 1987). In some *Megachile* species (leaf cutter bees) (Megachilidae), Dufour's gland compounds have been found mixed with larval food (bee bread), implying that they may serve as larval nutrition, or possibly to protect the larvae from fungal or bacterial attack (Cane and Carlson 1984, Duffield et al. 1984). Among short-tongued bees (Colletidae, Halictidae, Andrenidae and Melittidae) chemosystematic analysis suggests that Colletidae, Oxaeinae, Nomiinae and Halictinae comprise a monophyletic group (lactone producing bees), sharing macrocyclic lactones that are not found in any other hymenopterans. The other short-tongued bees (Andrenidae, Melittidae and Dufoureinae (currently Systrophinae)) form another monophyletic clade, sharing hydrocarbons (Cane 1983). The function of Dufour's gland in other solitary Apoidea like the Sphecidae (digger wasps), Crabronidae (sand wasps and bee wolves), and Ampulicidae (cockroach wasps) remains unexplored. Thus in addition to forming the cell lining, nest recognition seems to be another widespread function of Dufour's gland chemicals in bees. The same chemicals that are already in use for lining the nest surface can also be used by the organisms to identify their nests.

Various communicative roles of the Dufour's gland like kin recognition and nestmate recognition are reported in communally nesting species of Andrenidae and Colletidae, and social Halictidae (Fig. 3).

In the Apidae, the role of the Dufour's gland in producing brood cell lining is found in ground dwelling bees of the genera *Anthophora* (Anthophorini) and *Eucera* (Eucerini) (Stephen and Torchio 1961, Norden et al. 1980, Cane and Carlson 1984, Shimron et al. 1985) and in ground dwelling carpenter bees as well (*Xylocopa olivieri*, Apidae: Xylocopini) (Kronenberg and Hefetz 1984). Xylocopines generally nest in wood (mostly tree trunks), and ground nesting strategy is an exception for this group. Thus the Dufour's gland secretions may have secondarily gained the function of producing brood cell lining in xylocopines. The function of the Dufour's gland in secreting a cell lining in the various ground nesting Apidae can be the result of the retention of a function present in ancestors of this group who diversified to form the different present day phyla. However, cases of convergent evolution owing to similarity in ecological habits cannot be ruled out. Similar to its suggested role in the Megachilidae, the role of the Dufour's gland in producing larval food has been demonstrated in *Anthophora abrupta* (Norden et al. 1980). In carpenter bees (Xylocopini), Dufour's gland has been implicated in foraging site marking, i.e. marking flowers that have been visited for pollen or nectar collection (Fig. 3). This marking is temporary and fades away over a short period of time, over which the flower is able to replenish its nectar. In *Xylocopa virginica* (Xylocopini) Dufour's gland secretions are deposited on flowers visited by bees, and serves to deter visits by conspecifics on a short term (Frankie and Vinson 1977, Vinson et al. 1978). In kleptoparasitic Nomadini, male mandibular gland secretion has been found to mimic the Dufour's gland secretion of their hosts. Males have been found to coat females with their mandibular gland secretion, which renders the chemical profile of the female similar to the cell lining of their host, thereby facilitating kleptoparasitism (Tengö and Bergström 1977).

Among the corbiculate apids (Apini, Bombini, Meliponini and Euglossini), communicative functions for the Dufour's gland secretions have been reported in various taxa. In bumble bees (Bombini), the Dufour's gland has been found to increase in size and activity with the reproductive activity and age of queens, and queen worker differences in gland size have also been reported, implying a possible role as an egg marking pheromone (Abdalla et al. 1999). Queen signaling and nestmate recognition through Dufour's gland has also been implied (Tengö et al. 1991, Oldham et al. 1994, Ayasse et al. 1999). In *Bombus terrestris* it has been found that workers may advertise their sterility through esters secreted in their Dufour's gland, and thereby avoid being policed by queens or other egg laying workers. These esters disappear when an individual starts to develop ovaries (Amsalem et al. 2009, Amsalem and Hefetz 2010). Cuckoo bumblebees (subgenus *psithyrus*) can be attracted by the Dufour's gland odors of their hosts (other *Bombus*) to parasitize them (Fisher et al. 1993). In stingless bees (Meliponini) it has been found that reproductively active queens may have larger Dufour's glands than virgin queens and the gland may be absent in workers; however the function remains unknown and has been speculated as involvement in reproduction or in queen

signaling, and it has been suggested that the Dufour's gland secretions may act as a queen pheromone, as virgin queens have hydrocarbons in their Dufour's gland, while mated physogastric queens have esters in addition to hydrocarbons (Abdalla and Cruz-Landim 2004, Abdalla et al. 2004). In honey bees (Apini), there are multiple evidences for Dufour's gland esters functioning in pheromonal queen signaling. Such esters are initially absent in workers, but start being secreted in egg laying workers (Katzav-Gozansky et al. 2002, Oldroyd et al. 2002, Katzav-Gozansky et al. 2007, Malka et al. 2008). It has been proposed that multiple mating of queens triggers the production of esters from the Dufour's gland (Richard et al. 2011), while in the case of workers it is probably triggered by the absence of queen pheromone. The egg laying workers of *Apis mellifera capensis*, who act as social parasites of *A. mellifera scutellata*, produce Dufour's gland secretions that have been found to mimic the queen gland secretion better than that of egg laying *A. m. scutellata* workers, thereby increasing the efficiency of their social parasitism (Sole et al. 2002). The role of Dufour's gland in Euglossini remains unknown. Thus in honey bees (Apini), bumble bees (Bombini), and stingless bees (Meliponini) the Dufour's gland serves communicative functions like fertility signaling (queen pheromone) and nestmate recognition (Fig. 3).

The Vespoidea contains two major groups of social Hymenoptera: the Formicidae and Vespidae. Apart from these two families, the function of Dufour's gland remains unexplored in other Vespoidea (Tiphiidae, Sapygidae, Mutillidae, Pompilidae, Rhopalosomatidae, Scoliidae, and Sierolomorphidae). In ants (Formicidae), Dufour's gland secretions have often been found to act as a trail pheromone in Formicinae and Ponerinae (Law et al. 1965, Hölldobler and Wilson 1970, Williams et al. 1981, Bestmann et al. 1995, Blatrix et al. 2002, Jeanson et al. 2003). They have a recruitment effect, serve in territorial marking, or in setting foraging direction (Cammaerts et al. 1977, Greene and Gordon 2007). Often the Dufour's gland acts in unison with other glands, like the poison gland, in communicating recruitment signals for recruitment of workers to food or nesting sites. Secretions of the Dufour's gland are often applied on the substrate by extruding the sting and dragging it (Hölldobler and Wilson 1970, Morgan 2009). However, other glandular sources of trail pheromone originating in the metasoma (hindgut, pygidial gland, post-pygidial gland) or legs (tibial glands, footprint glands on the hind pre-tarsi) are also known (Morgan 2009). The Dufour's gland secretion can have colony specificity and species specificity, thereby ensuring fidelity of the trail (Traniello 1980, Haak et al. 1996). The Dufour's gland has also been implicated as a storage site for chemicals involved in the alarm-defense system of ants, whereby the same chemicals can produce an alarm reaction among nestmates, while simultaneously acting as repellents to intruders (Wilson and Regnier 1971, Whitehouse and Jaffe 1996). In slave making ants of the genus *Polyergus* Dufour's gland chemicals have been found to be used in manipulating the behavior of the host species. Dufour's gland compounds facilitate the new queen of the slave making ant to invade a host colony, and reduce any aggression shown by the host workers towards the usurper (Topoff et al. 1988, Mori et al. 2000). Such compounds may also act as a repellent and thereby facilitate usurpation of a host colony (Ruano et al. 2005). Another function of

Dufour's gland in slave making ants is to act as a source of propaganda substances that elicit panic among defending host workers, thereby increasing the efficiency of slave capturing raids (Brandt et al. 2006). Dufour's gland compounds have also been found to act as sex pheromones, and can also be involved in the calling behavior exhibited by females, whereby virgin gynes place themselves in strategic positions outside their nests and "call" for mates by releasing sex pheromones (Hölldobler and Wust 1973, Hölldobler and Wilson 1990, Ayasse et al. 2001).

In the hover wasps (Vespidae: Stenogastrinae) the Dufour's gland substances act as a substrate on which eggs are laid and food is placed for subsequent consumption by larvae and adults (Sledge et al. 2000, Fortunato and Turillazzi 2012). The secretion of a jelly-like substance by the Dufour's gland that functions in larval nutrition has been proposed as an important step towards the evolution of sociality in this lineage. It enables anchoring eggs and larvae, and the storage of liquid food in the nest, subsequently facilitating the evolution of behavioral mechanisms that facilitate social interaction (Turillazzi 1989, Cervo et al. 2002). Apart from these functions, Dufour's gland secretions also serve as ant repellents. They are used to construct sticky barriers which serve as ant guards around the nest (Sledge et al. 2000, Fortunato and Turillazzi 2012). These aid in preventing predation of immature brood by ants. Dufour's gland compounds may also function in nestmate recognition (Cervo et al. 2002). Additionally, the Dufour's gland secretions serve as food for both larvae and adults and can be stored for future consumption. This seems to be an important development that may have facilitated the evolution of sociality in this group (Turillazzi 1989, Cervo et al. 2002, Fortunato and Turillazzi 2012). The Dufour's gland secretions function as ant repellants (Sledge et al. 2000), and this should be another important factor facilitating progressive provisioning of brood. Progressive provisioning of brood is a character shared by most eusocial Hymenoptera, and is important for the evolution of sociality (Hunt 2007). Evidence for Dufour's gland compounds acting in nestmate recognition in stenogastrines reinforces the idea of chemicals having other primary functions being secondarily involved in chemical communication. Since the gland secretions have to be applied inside cells, and also outside and around the nest, to repel ants, this should facilitate nest or nestmate recognition by nest odor. Thus the Dufour's gland secretions can be intertwined with the coevolution of brood rearing, avoiding brood predation, and maintaining colony fidelity, and appear to have an important role in the evolution of sociality in hover wasps.

Among other vespids, in the Vespinae the Dufour's gland has been postulated to facilitate social parasitism by increasing acceptance of parasites among host individuals, or by acting as an alarm pheromone, eventually serving as a means of usurping and controlling the host colony (Jeanne 1977, Reed 1982, Reed and Akre 1982). The Dufour's gland secretion of *Vespa orientalis* has been reported to be slightly lethal to honey bees, thereby implicating a role in venom secretion (Barr-Nea et al. 1976). This may be another example of an adaptation of Dufour's gland secretions for a function not reported in other hymenopteran taxa. In Polistinae, Dufour's gland compounds have been implicated in dominance interactions, egg marking, nestmate recognition,

and queen signaling (Downing and Jeanne 1983, Downing 1991, Dani et al. 1996a, 1996b, Mitra et al. 2011, Mitra and Gadagkar 2011, Mitra and Gadagkar 2012a, 2012b). In the polistine wasp *Ropalidia marginata* the Dufour's gland has been shown to be involved in producing the queen pheromone, using which the queen conveys her presence to workers and thereby maintains reproductive monopoly (Mitra et al. 2011). It has also been found that the Dufour's gland hydrocarbon composition varies as a function of ovarian development, thereby suggesting that such chemicals can act as an honest signal of the queen's fertility (Mitra and Gadagkar 2011, Mitra and Gadagkar 2012a, 2012b). Interestingly it has been found that the cuticle and the haemolymph of *R. marginata* also contain the same set of hydrocarbons as found in the Dufour's gland, thereby implying that these hydrocarbons can be synthesized in the oenocytes of fat bodies, from where they enter circulation in the haemolymph and are finally sequestered and stored in the Dufour's gland or secreted on the cuticle (Mitra and Gadagkar: in press). The function of the Dufour's gland remains unexplored in other Vespidae like potter wasps (Eumeninae) or pollen wasps (Masarinae). Examples of chemical communication through Dufour's gland in vespids, like dominance signaling, queen signaling, nestmate recognition or social parasitism (Fig. 3), again suggest adaptations in a case specific manner. The ability to perceive chemicals secreted from the Dufour's gland should be necessary to facilitate the evolution of adaptive communicative functions. Interestingly, in a polistine wasp it was found that dominance signaling through Dufour's gland secretions may act via egg marking (Downing and Jeanne 1983, Downing 1991), perhaps exemplifying the ancestral function of egg coating giving rise to novel communicative roles. The role of the Dufour's gland in communication in vespids may have coevolved with the evolution of various behavioral and chemical communication systems exhibited by vespids, or it may also have evolved coincidentally with the evolution of vespid sociality.

Concluding remarks

It can be seen that the Dufour's gland secretion has undergone a variety of shifts in its functionality in various hymenopteran lineages. The ancestral function related to egg coating and attachment of eggs to the substrate might be retained (or perhaps secondarily gained) only in stenogastrines, in addition to a few extant clades like ichneumonids and perhaps a few polistines (Fig. 3). In parasitic wasps, it has gained the function of host marking, which may be the most widespread function of the Dufour's gland in non-aculeates (Fig. 3). Dufour's gland secretions form a chemical mark on the host body, which can be used by the parasites as a recognition cue, thereby preventing over-infection of the same host. In these groups, the ovipositor is long, sting-like, and the precursor of the sting of Aculeata, and thereby secretions of the Dufour's gland (perhaps involved in oviposition and other reproductive functions) can get co-opted to be used as semiochemicals used to avoid oviposition in a host that is already parasitized. This is particularly interesting from the perspective of evolution of semiochemicals, be-

cause chemicals that already exist for other purposes often get co-opted later for being used as a signal (Stoka 1999). Since the Dufour's gland chemicals may be involved in oviposition, they will inadvertently get deposited on the host when the parasite injects her eggs. With the evolution of the signaling system, which requires developing the ability to detect these chemicals, these chemicals can advertise the unavailability of a host to other potential parasites. The Dufour's gland secretions have also been reported to act as sex pheromone in braconids (Fig. 3). Unsaturated hydrocarbons have been reported in Dufour's gland secretions of braconids and ichneumonids (Fig. 2), and it is possible that the ability to secrete such semi-volatile compounds is a necessary prerequisite for evolving the function of sex pheromones or other semiochemicals that are detected at a distance. The presumed ancestral role of involvement in oviposition or egg coating appears to have been retained in ichneumonids (Huang et al. 2008), but it may well be that such a role is present in other taxa as well, awaiting discovery.

In some non-apid bees (Colletidae and Halictidae), the gland has primarily acquired the role of producing cell linings, which are essential for preserving provisions for the brood. This clearly has arisen in response to the challenges faced for keeping food unattended and unspoiled in cells constructed in the soil, where food is always prone to becoming over-hydrated and being attacked by microbes. The chemistry of Dufour's gland secretions have been altered accordingly in these two groups of ground nesting bees and consist of terpenoid esters and other esters, and macrocyclic lactones in addition to hydrocarbons (Fig. 2). These chemicals often form a natural polyester lining inside the brood cells, acting as an effective water repellent. It is possible that this function of producing the brood cell lining from Dufour's gland secretions may have arisen simultaneously with diversification of the ground nesting bees into their present state. Interestingly, there are also ground nesting basal melittids and megachilids that do not line their cell walls (Cane 1983). Hence it is possible that this trait has arisen independently multiple times. The function of producing cell lining from Dufour's gland secretions is again found in ground nesting apid bees (Anthophorini and Eucerini), although the chemistry of the secretions is different, comprising of hydrocarbons and esters (Fig. 2). The function of producing brood cell lining is lost in social bees of Apini, Bombini and Meliponini, where communicative functions take precedence, and unsaturated hydrocarbons and esters seem to be involved in this function. Members of Apini, Bombini and Meliponini generally construct their nests from wax produced from abdominal wax glands (cuticular exocrine glands), sometimes also using cellulose and other material collected from their surroundings in the nest construction process. Hence it is not surprising to find that the Dufour's gland no longer plays a role in lining their cells or producing material for nest construction. Communicative roles are also seen in other social bees like the Halictidae, and in various vespoids. The Dufour's gland may have played an important part in the evolution of sociality in stenogastrines, as the conditions necessary for successful social living would have to be fulfilled in other ways in the absence of the Dufour's gland. The ancestral function of attachment of eggs to the substrate through Dufour's gland secretions may also have been retained in stenogastrines (however secondary gain of such function cannot be ruled out). The Dufour's gland plays communicative roles in polistines as well, emphasizing

ing the utility for developing modes of chemical communication in conjunction with the evolution of social life. In the Formicidae there is enormous variation in the communicative roles played by the Dufour's gland, concomitant with the wide ranging adaptive radiation and speciation undergone by this group. The Formicidae show appreciable variation in communicative roles of the Dufour's gland ranging from trail pheromone and territory marking to alarm-defense, slave making, and serving as sex pheromones (Fig. 3). The role of the gland in trail formation has been demonstrated in many trail forming species. The ability of the ants to perceive Dufour's gland chemicals should be a prerequisite for involvement of such chemicals in trail marking. Accordingly the chemical composition of the Dufour's gland also includes various volatile and semi-volatile compounds like unsaturated hydrocarbons, terpenoids, alcohols, acids, quinones and aromatic compounds. The involvement of the Dufour's gland in the alarm-defense system or in slave making, may be secondary adaptations. Perhaps the involvement of Dufour's gland chemicals as an alarm pheromone has been exploited by slave making species to give rise to this special kind of social parasitism. The involvement of the Dufour's gland in sex pheromone production in ants is reminiscent of a similar role in non-aculeate hymenopterans.

Thus the Dufour's gland secretions display a fair degree of functional plasticity, in conjunction with the diversity of ecological habits followed by different groups of Hymenoptera (Fig. 3). The basic function of the gland in all Hymenoptera remains the secretion of chemicals, but the nature of secretions and the function of the secreted chemicals have undergone appreciable modification in various taxa. This illustrates how an organ can evolve to give rise to various functions in response to challenges posed by the way of life followed by a lineage. The function of the Dufour's gland remains unexplored in various basal Hymenoptera, especially the Symphyta. Future explorations in that direction should be able to provide a clearer picture of the role of the gland in the ancestral condition. This will result in a comprehensive view of the evolution of Dufour's gland function in the Hymenoptera.

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